100 Identification algorithms for *Burkholderia cepacia* complex bacteria

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Burkholderia cepacia complex (BCC) organisms are important cystic fibrosis (CF) pathogens with a considerable influence on morbidity and mortality. Presently, nine BCC species have been described but ongoing collaborative studies recently revealed the existence of at least another 6 new BCC species occurring in CF samples. Identification of all of these BCC species is a complicated challenge requiring a combination of multiple molecular diagnostic procedures and is mostly restricted to reference centers.

The algorithm used for the identification of BCC isolates in our reference center is outlined and is applied to examine a number of BCC isolates representing several additional BCC bacteria not belonging to one of the established species. An overview of current identification approaches for BCC bacteria is presented.

101* Survival of Cepacia Syndrome

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Cepacia Syndrome is a rapidly progressive and usually fatal pneumonia mostly due to infection with Bcc genomovars III or II. It is characterised by a rapidly progressive respiratory illness, pyrexia, elevated leukocyte count, progressive and confluent CXR changes and positive blood cultures.

A 10 year old girl presented with a respiratory exacerbation in May 2006. Sputum had previously grown Alcaligenes spp. Due to multiple severe allergies she was desensitised to ceftazidime and began a 2 week course of IV ceftazidime and tobramycin. She responded to treatment with an improvement in RFTs and symptoms. Sputum on this admission grew Burkholderia cenocepacia IIIA. She had a further respiratory exacerbation in August and the same organism was re-cultured from sputum. For this episode she was treated with IV tigecycline. In November she presented with an increase in cough, weight loss, profound lethargy, SpO_2 of 92%, generalised coarse crackles and pyrexia of 40ºC. Investigations revealed neutrophils 17.3, CRP 121 and new bilateral infiltrates on CXR. Bc. Gv IIIA was isolated in sputum and blood cultures. Treatment included prednisolone 40 mg daily and IV tobramycin, meropenem and ceftazidime. Six days into treatment she began to improve. She completed 21 days of IV antibiotics and steroids. She continues with a productive cough and increased requirement for intermittent IV antibiotics. There are very few reports of patients surviving cepacia syndrome. The early use of steroids may have contributed to survival by reducing the influx of proinflammatory cytokines and neutrophil induced inflammation associated with cepacia syndrome.

102 Morbidity of a peadiatric population with CF, according to chronic *P. aeruginosa* (Pa) colonization

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Monitoring of respiratory infection, identification of morphotypes of Pa and chronic infection evaluation are important to determine prognosis and choose the treatment. To describe the situation of Pa respiratory infection and evaluate the chronic colonization.

The study was made in a pediatric reference center for CF and involved 116 patients. Respiratory cultures collected between Jan-Dec 2006 were included. Sex, age, number of exams/patient/year, cultures positivity, number of patients with positive cultures to Pa, bacteria morphologic types, and chronic colonization criteria were observed. Data related to frequency were analyzed.

There were a prevalence of 55% in females, the mean age was 9 yo and 10 mo and median age: 9 yo and 5 mo (min 11, max 264 mo). 455 cultures of respiratory secretion were made, with average of 3.92 cultures/patient/year. The vast majority of this cultures were positive: 348/455 (76.5%). From 116 children and teens with CF, 66 (57%)were positive to Pa, in which 42 (63.6%) made 4 or more cultures/year and therefore were evaluated about chronic colonization (CC): 20/42 (47.6%) – yes. The median age in this group was 8 yo and 4 mo. In the group of children that were positive to Pa, 17/66 (25.7%) showed only non-mucoid morphotype, 20/66 (30.3%), mucoid and 29/66 (44%), both of them.

The early isolation of Pa, the high prevalence of mucoid morphotype and chronic colonization show the severity of the disease in this group of pediatric patients. These considerations reassure the importance of including CF in the investigation of respiratory disorders. Microbiology aspects should be correlated to image and functional presentation and therefore contribute to treatment approach and prognosis.

103 Genotype diversity of *P. aeruginosa* in CF sputum samples

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Introduction: Longitudinal studies have shown that patients with CF may harbour more than one strain of *P. aeruginosa*, but few studies have examined multiple colonies from single sputum samples. We aimed to study the diversity, according to genotype, of *P. aeruginosa* in single sputum samples.

Methods: Sputum samples from 16 patients with CF, infected with *P. aeruginosa* for at least 12 months, were treated with sputasol then serially diluted and incubated for 12hr at 37ºC on the following agar plates: BHI agar, *P. aeruginosa* isolation agar (PIA), and PIA with ciprofloxacin (2 mg/l), ceftazidime (8 mg/l), tobramycin (8 mg/l), and colistin (4 mg/l). 47 colonies from each sputum were picked from the overnight culture, including representatives from each antibiotic plate and each morphotype. The colonies were typed using multilocus sequence typing (MLST).

Results: Demographics were: 9 were male, mean age 28.3 yr, mean FEV1 49.8% predicted. Full MLST data were obtained for 6–39 colonies (mean 26.8) per sample. 8 patients had only 1 genotype, 6 had 2 genotypes, 1 had 3 genotypes, and 1 had 4 genotypes. In 4 patients different morphotypes were associated with different genotypes, in 1 patient mucoid and non-mucoid colonies had different genotypes, and in 5 patients particular antibiotics selected for particular genotypes. Overall 50% of patients harboured more than one strain, which was similar to results from studies testing fewer colonies with PFGE or RAPD genotyping.

Conclusions: In the first study to genotype more than 12 colonies per sputum sample or use MLST, we found that patients harboured between one to four strains and 50% of patients harboured more than one strain.